The impact of the dietary supplement N-carbamylglutamate on the biochemical and morphological aspects of blood in lactating cows investigated

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Abstract. The aim of this study was to investigate the effect of N-carbamylglutamate supplements on protein metabolism and nitrogen utilization in lactating cows. The findings revealed that the total protein level in the experimental group was significantly greater than in the control group ($p<0.05$), suggesting an improvement in dietary nitrogen utilization efficiency. Additionally, albumin levels were increased in the experimental group, indicating enhanced protein synthesis and utilization. Ammonia levels were significantly lower in the experimental group ($p<0.05$), demonstrating enhanced metabolic utilization of ammonia nitrogen. Urea levels were also lower in this group ($p<0.05$), implying increased use of endogenous urea for microbial protein production. Creatinine levels, on the other hand, were higher in the experimental group ($p<0.05$), reflecting an increased utilization of ammonia nitrogen in the synthesis of nitrogenous compounds

1 Introduction

In the digestive process of ruminants, the breakdown of proteins leads to the production of ammonia as a metabolic byproduct [6]. This ammonia, if accumulated excessively, poses a risk to cell viability, particularly in highly productive lactating cows. Its adverse effects include alterations in blood pH, interfering with oxygen transport and potentially inducing cellular hypoxia [7, 9]. Additionally, excess ammonia can disrupt cellular functions by competing with essential ions such as Na+ and K+ [17].

The analysis of literature on the subject confirms the hypothesis regarding the effectiveness of N-carbamylglutamate (NCG) use in dairy cattle farming [1, 5]. For instance, there is evidence for the successful application of the ornithine activator in sheep, sows, weaned pigs, and broiler chickens [8, 11, 14, 16]. The addition of this dietary supplement to the feed has yielded positive results in terms of production and product quality, according to several authors. Additionally, these activators have been proven to

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increase milk yield and composition in cows [10]. Some authors have also noted that activation of the ornithine pathway promotes steroidogenesis in vitro for bulls and boars [2-3]. N-carbamylglutamate, a synthetic analogue of N–acetylglutamate, has proven to be an especially promising compound in this context.

The activation of the ornithine cycle through increased endogenous arginine synthesis and ammonia utilization could be considered a potential approach for optimizing metabolic processes in animals. N-acetylglutamate (NAG) and its synthetic analogue N-carbamylglutamate (NCG), which serve as catalysts for the ornithine cycle, have been identified as possible compounds that could enhance the activity of enzymes involved in this cycle [5, 12]. NCG, a biologically stable and structurally similar compound to NAG, has been shown to stimulate the activity of carbamoyl phosphate synthase, an essential enzyme for the formation of carbamoyl phosphate within the ornithine pathway. Carbamoyl phosphate then participates in subsequent steps of the pathway, leading to the production of argininosuccinate and urea. The use of N-carbamoyl-L-glutamate (NCG) may help to enhance the activity of the ornithine cycle, which could benefit animals under conditions of stress, disease, or increased demand for nitrogen. This could optimize nitrogen metabolism and improve metabolic efficiency [13]. Despite the extensive research on NCG, its effects on the rumen microbiota and gastrointestinal tract remain largely unexplored, and further investigation into these aspects could yield valuable insights. In summary, NCG supplementation could be a promising approach to optimizing metabolic processes in animals and potentially improving productivity.

2 Materials and methods

Two distinct groups were established for the study, namely an experimental group and a control group. Both groups were subjected to the standard diet regimen typically implemented on the farm (n=20). This standard diet is a well-established nutritional protocol utilized for maintaining the health and productivity of the animals within the farm's operations.

The experimental group, comprising one of the two groups under investigation, received the standard diet supplemented with N-carbamylglutamate (NCG), a synthetic analog of N–acetylglutamate (NAG). This dietary modification aimed to assess the potential effects of NCG supplementation on various physiological parameters, particularly those related to protein metabolism and nitrogen utilization.

In contrast, the control group, serving as a comparative baseline, received the standard diet without any additional supplementation. This control group allowed for the evaluation of the specific impacts of NCG supplementation by providing a reference point against which to measure changes observed in the experimental group.

Establishing both experimental and control groups under identical dietary conditions, except for the presence of NCG supplementation in the experimental group, ensured a controlled experimental design. This approach enabled the isolation of the effects of NCG supplementation from other potential confounding factors, such as differences in diet composition or management practices.

By utilizing this controlled experimental setup, the study aimed to elucidate the specific effects of NCG supplementation on the parameters of interest, providing valuable insights into its potential as a nutritional intervention to optimize metabolic processes in the animals. N-carbamylglutamate (NCG) was added to the main ration of the experimental group at a dosage of 20 g/head/day continuously for 30 days. The control group received the same main ration without NCG supplementation.

To evaluate the effectiveness of NCG usage, results obtained before the experiment and at two study periods (14 and 30 days) before its conclusion were compared. This approach

ensures that observed changes are attributed to NCG supplementation, minimizing the influence of other factors.

Biochemical blood analysis was conducted using an automatic biochemical analyzer ERBA XL (ERBA XL 100). Ammonia levels were analyzed using the Sentinel "Ammonia ultra" kit (Italy), while hematological parameters were determined using a Class 3 analyzer (Mindray BC-2800 vet).

A holistic approach to data analysis makes the results more trustworthy and relevant for practical use in animal husbandry.

3 Results and Discussion

During the analysis of the acquired data, it was determined that the quantitative indicators of blood cell elements corresponded to those of clinically healthy animals in both the experimental and control groups and were within the physiological ranges of a healthy, producing animal. Based on the findings presented in the table, no significant discrepancies were identified between the experimental and control groups.

According to the results of the study, the effect of N-carbamylglutamate (NCG) on the biochemical profile in lactating cows was noted. The experimental group, which received NCG supplementation, showed notable changes in various parameters compared to the control group, indicating improved protein metabolism and nitrogen utilization. Firstly, the total protein concentration in the experimental group was consistently higher throughout the study compared to the control group ($p<0.05$). This increase in total protein levels reflects enhanced efficiency in nitrogen utilization in the experimental group, indicating improved protein metabolism. Additionally, the level of ammonia in the blood was significantly lower in the experimental group compared to the control at both time points ($p<0.05$). This reduction in blood ammonia levels suggests increased metabolic utilization of ammonia nitrogen, indicating a more efficient nitrogen metabolism. Efficient nitrogen metabolism, with the aim of minimizing the negative effects associated with ammonia accumulation, was achieved through the introduction of a nitrogen-containing compound (NCG). Despite this, the lactation level in cows in the experimental group remained stable at its initial level, while a decline was observed in the control group. This suggests that NCG supplementation did not adversely affect milk production. Furthermore, the level of urea in the blood of the experimental group was notably lower by 14.9% and 10.4%, at the two study periods, compared to the control group ($p<0.05$), indicating increased utilization of endogenous urea for microbial protein synthesis in the rumen, further supporting the improved nitrogen utilization with NCG supplementation. Additionally, the dynamics of creatinine levels in blood demonstrated a higher level in the experimental group by 4.5% and 3.7%, at the two study periods compared to the control group ($p<0.05$), suggesting an increased utilization of ammonia nitrogen for creatinine synthesis.Nitrogen-containing compounds, which reflect improved protein turnover and utilization, have been found to positively influence protein metabolism, nitrogen utilization, and lactation performance in highly productive cows when supplemented with N-carbamylglutamate. These findings provide valuable insights into the potential benefits of this supplementation in optimizing protein nutrition and overall productivity in dairy herds.

Table 1. The effect of the NCG supplement on the cellular and biochemical composition of the cows' blood (M±m, n=20).

Parameters	Experimental group			Control group		
	The beginning of the experiment	14 days	30 days	The beginning of the experiment	14 days	30 days
Hematological parameters						
Erythrocytes,10^{12}/l	6.9±0.3	7.3±0.4	7.2±0.4	7.0±0.2	6.9 ±0.4	7.0±0.3
Hemoglobin, g/l	110±5	115±3.6	110±4.5	111.3±6.7	105.2±6.7	108.4±4.3
Leucocytes,10^{9}/l	10.2±	10.8±	10.5±	11.4±	11.1±	11.5±
Platelets,10^{9}/l	300±60	310±40	295±47	280±55	300±30	330±41
Hematocrit, %	34±2.3	34±1.4	34±1.8	34±2.2	34.8 ± 1.5	34±1.3
Biochemical parameters of blood serum:						
Total protein, g/l	77.12±0.6	84.4±0.2*	75.7±0.4	76.6±0.5	79.6 ± 0.4*	77.7±0.3
Urea, mM	5.5±0.3	4.34±0.2*	4.71±0.3	4.95±0.7	5.29±0.2*	5.2±0.2
Creatinine, mcM	74.5±2.4	81.5±1.8*	80.0±1.4	76.3±3.1	78.1±1.1*	77±2.1
Ammonia, mM	108.4±3.1	101.68±4.2*	100.2±3.7*	115.5±4.1	129.70±5.6*	120.2±4.8*
Albumins, g/l	29.1±4.1	37.1±2.7*	31.4±1.1*	28.5±2.4	28.73±1.2*	27.2±0.7*
Globulins, g/l	48.02±0.3	46.04±0.5	44.3±0.4	48.04±0.5	50.78±0.4	50.46±1.2
Glucose, mM	3.26±0.3	3.37±0.4	3.3±0.1	3.16±0.2	3.07±0.3	3.1±0.2
Cholesterol, mM	2.8±0.4	3.3±0.2	3.6±0.4	2.5±0.3	2.8±0.4	3.8±0.5

Note: *P<0.05, according to the t – criterion when compared with the control

4 Conclusion

The study provides a comprehensive assessment of the effects of N-carbamylglutamate (NCG) supplementation on several physiological and biochemical indicators in high-producing lactating dairy cows. The findings emphasize the positive effects of NCG on protein metabolism, nitrogen utilization, and overall milk production, offering valuable insights into optimizing dairy herd efficiency.

Analysis of blood cell counts revealed that both experimental and control animals maintained values within normal ranges for clinically healthy cows, indicating that NCG administration did not negatively impact the overall health status of the animals.A key finding of this research was the significantly higher total protein concentration observed in the experimental group, when compared to the control group ($p<0.05$). This finding suggests an improvement in nitrogen utilization and protein metabolic efficiency as a result of NCG supplementation.

The elevated protein levels observed in the experimental group indicate a more effective integration of dietary nitrogen into bodily protein, which is essential for maintaining high milk production levels. This finding has significant implications for the dairy industry, as it suggests that NCG supplementation may be a valuable tool for enhancing protein metabolism and milk production in dairy cows. The study also demonstrated a significant reduction in blood ammonia levels in the experimental group ($p<0.05$). This suggests an improved metabolic utilization of ammonia nitrogen, which is crucial for reducing the potential toxic effects of ammonia accumulation. Additionally, the lower blood urea levels in the experimental group, indicate that NCG promotes the utilization of endogenous urea for microbial protein synthesis in the rumen. This enhanced utilization of urea further supports the observed improvement in nitrogen metabolism with NCG supplementation. The experimental group demonstrated statistically significant increases in blood creatinine levels, respectively, compared to the control group at the two study time points ($p<0.05$).

These findings suggest a higher utilization of ammonia nitrogen for the synthesis of creatinine, which is an indicator of increased protein turnover and utilization.

Future research should focus on longer-term studies to better understand the mechanisms underlying NCG's effects and establish optimal supplementation strategies for different stages of lactation. In conclusion, NCG supplementation holds promise as a strategy to improve nitrogen metabolism and protein utilization, leading to enhanced lactation performance and productivity in dairy cows. These findings contribute to the advancement of more efficient nutritional approaches in the dairy industry with the aim of achieving higher milk production while maintaining sustainability.

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